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ACE and ACE2: their role to balance the expression of angiotensin II and angiotensin-(1–7)

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The discovery of angiotensin-converting enzyme 2 (ACE-2) has revealed a far more complex enzymatic cascade that may influence the renin-angiotensin system within the kidney, specifically the expression of the functional products angiotensin II (Ang II) and Ang-(1-7). The regulation of this critical system involved in blood pressure control must now encompass the integral relationship of ACE and ACE-2 activities.

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In this issue, Tikellis *et al.*[1] document the developmental expression of both angiotensin-converting enzyme (ACE) and its novel homologue, angiotensin-converting enzyme 2 (ACE2), in the kidneys of the normotensive Wistar Kyoto (WKY) rat strain and the spontaneously hypertensive rat (SHR). These studies build on the original report by Penninger and colleagues, who found that reduced expression of renal ACE2 in three distinct models of hypertension correlated with the hypertensive phenotype.[2] In that study, the ACE2-null mice expressed higher circulating and cardiac tissue levels of angiotensin II (Ang II). Moreover, our preliminary data demonstrate that ACE2 is the predominant pathway for the metabolism of Ang II in the murine heart, providing additional evidence that ACE2 is a key component of the tissue renin–angiotensin system (RAS) (PJ Garabelli *et al.*, *Hypertension* 2003; **43**: 1349, abstr.). ACE, a zinc metalloendopeptidase that functions as a carboxyl-directed dipeptidase, converts Ang I to Ang II and also inactivates bradykinin. ACE2 is the first known homologue of ACE, exhibiting over 60% sequence similarity to ACE.[3,4] Unlike ACE, ACE2 is resistant to ACE inhibitors, and ACE2 catalytic activity is exerted by cleavage of a single amino acid residue at the carboxyl terminus. Although ACE2 was first shown to cleave Ang I to Ang 1–9 given its close homology to ACE,[3] subsequent studies revealed that Ang II exhibits a far greater catalytic rate than Ang I.[5] To date, only Li *et al.*[6] have found that Ang I may be a preferred substrate for ACE2 in proximal tubules from rat kidney, as, in their study, they found no evidence for ACE2-dependent generation of Ang 1–7 from Ang II. The inability of the proximal epithelial ACE2 to cleave Ang II to Ang 1–7 is surprising particularly given the lack of evidence for ACE2 isozymes. Moreover, we and others find significant ACE2 activity with the use of high-performance liquid chromatography separation to quantify the conversion of Ang II to Ang-(1–7) in the membrane fraction of the rat renal cortex or renal homogenates.[7,8] The issue of whether ACE2 participates in the tubular processing of Ang II and Ang-(1–7) is an extremely important one given that the proximal tubules contain the highest density of ACE2 and that these peptides exhibit different actions within the kidney.[9]

In the study by Tikellis and colleagues,[1] the developmental expression of ACE2 was compared with that of ACE. The genesis of hypertension in the SHR is still not resolved but most likely involves the RAS, and the critical period for the development of the hypertension is approximately 5–7 weeks of age. Indeed, blockade of the RAS by ACE inhibition or Ang II type 1 receptor (AT_1 receptor) antagonists is known to have a long-lasting effect on blood pressure that exceeds the period of treatment. Tikellis *et al.*[1] demonstrate that, in comparison with the WKY strain, ACE2 mRNA and enzyme activity was increased in 1-day-old SHR neonates, but reduced at 42 and 80 days. The predominant expression of ACE2 in both strains was found in the proximal-tubule elements; however, immunocytochemical staining for ACE2 was also evident in the glomerulus, and the extent of staining was increased in the SHR. Although a reduction in renal ACE2 may conceivably contribute to an elevation in blood pressure in the SHR, the authors also found that renal ACE mRNA and activity were significantly reduced in both the neonate and the adult SHR. Thus, the issue of whether a shift to lower ACE2 in the presence of reduced ACE activity contributes to the hypertensive phenotype remains to be investigated. Although it is plausible that reduced ACE2 gene expression will favor increased levels of Ang II through reduced conversion into Ang-(1–7), it is necessary to also consider that ACE is the primary enzyme involved in the inactivation of Ang-(1–7).[10] Measurements of renal tissue concentrations of both Ang II and Ang-(1–7) will be required to answer this question. Moreover, it is important to emphasize that ACE and ACE2 probably function in concert within tissues to constitute an influential point in the actions of the RAS pathway, as proposed recently by Ferrario *et al.*[11] As is mentioned above, a reduction in ACE, although depleting Ang II as a substrate for ACE2 to be subsequently converted to Ang-(1–7), will increase the overall level of Ang I, which is directly converted to Ang-(1–7) by the action of tissue-specific endopeptidases.[12] Until an age-dependent correlation of the change in renal content of Ang II and Ang-(1–7) is obtained, it will not be possible to discern the functional implications of the alterations in ACE and ACE2 that were observed by Tikellis *et al.*[1] Certainly, the significance of the current findings is

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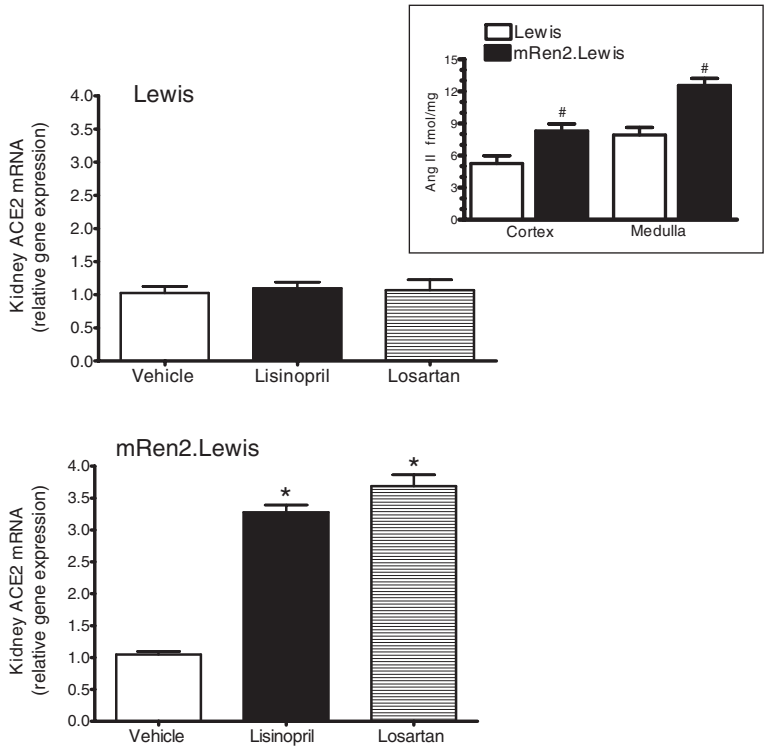

Figure 1 | Differential response of renal angiotensin-converting enzyme 2 mRNA expression to renin–angiotensin system blockade in normotensive and hypertensive rats. Renal angiotensin-converting enzyme 2 (ACE2) mRNA levels were determined in the renal cortex of normotensive Lewis (upper panel) and hypertensive mRen2.Lewis (lower panel) rats after a 2-week treatment with either the ACE inhibitor lisinopril (20 mg per kg per day) or the angiotensin II type 1 receptor antagonist losartan (10 mg per kg per day) (CMF *et al.*, unpublished observations). Inset: Cortical and medullary tissue levels of angiotensin II (Ang II) in the Lewis and mRen2.Lewis rats. $^{*}P < 0.05$ versus vehicle ($n = 8$ per group); $^{\#}P < 0.05$ versus Lewis ($n = 5$ per group).[18]

further complicated by the fact that at least a fraction of renal Ang II and possibly Ang-(1–7) is derived from receptor-mediated uptake of blood-derived peptides.[13,14] The extent to which ACE2 participates in the catabolism of sequestered Ang II from the circulation is also unknown.

Finally, one must acknowledge that the reliance on the measurement of enzyme activity with synthetic substrates precludes a direct comparison of ACE and ACE2 activities. Although fluorescent substrate assays provide a sensitive and efficient means to quantify activity, provided adequate controls are used, the ACE2 substrate is not entirely specific for ACE2, as other peptidases may cleave this substrate. Indeed, the study by Tikellis *et al.*[1] emphasizes the inclusion of the prolyl oligopeptidase inhibitor z-pro-prolinal to prevent hydrolysis of the Pro–Lys bond of the substrate. The use of the non-peptide ACE2 inhibitor MLN-4760 as utilized in the present study ensures specificity of the fluorescent substrate in contrast to the general metalloprotease inhibitors such as EDTA or *o*-phenanthroline. The DX-600 compound is also a potent ACE2 inhibitor; however, this peptide is likely not to be resistant to proteolysis in tissue homogenates, and the resultant fragments may exhibit additional inhibitory activity against other peptidases, which may explain the antihypertensive actions of this agent in the SHR.[15] Although there are no technical issues with the assays used by Tikellis *et al.*,[1] and both activity and mRNA levels closely match, the comparative activities of ACE and ACE2 in the kidney cannot be ascertained with the current approach. At this point, the use of the endogenous substrates Ang I and Ang II assayed under identical conditions will provide an accurate assessment of the balance of both activities in the kidney and other tissues. In this regard, both ACE and ACE2 were increased in the lungs of nutritionally deprived rats that exhibit higher blood pressure; however, ACE activity was 100-fold greater than ACE2 activity (rate of Ang-(1–7) produced),[8] and it is difficult to conclude that ACE2 would effectively buffer the changes in pulmonary ACE in this model of fetal programming.

Although Tikellis *et al.*[1] take the viewpoint that ACE and ACE2 constitute a counter-regulatory mechanism within the kidney, their results reveal a somewhat similar downregulation of the two enzymes in the WKY rat and the SHR during development. This scenario is of interest in light of our own studies that first revealed that Ang II, through the AT_1 receptor, appears to negatively regulate ACE2 expression in the heart, kidney, and astrocytes.[7,16,17] The overall regulation of ACE2 may be more complex than anticipated given the pronounced differences in ACE2 mRNA expression within the kidneys of the normotensive Lewis (Figure 1, upper panel) and hypertensive mRen2.Lewis (Figure 1, lower panel) rats following the ACE inhibitor lisinopril or the AT_1 receptor antagonist losartan. The inset panel of Figure 1 illustrates that both cortical and medullary levels of Ang II are elevated in the mRen2.Lewis rat and suggests that blockade of an increased RAS results in an enhanced response in the expression of ACE2. Indeed, the increase in ACE2 is consistent with elevated levels of Ang-(1–7) following AT_1 receptor or ACE blockade, as well as the functional contribution of Ang-(1–7) to the blood pressure-lowering effects of these agents.[17] Elucidation of the regulatory mechanisms for ACE2, and the functional aspects of the enzyme in distinct tissue compartments, remain two key areas of continuing study.

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REFERENCES

1. Tikellis C, Cooper ME, Bialkowski K *et al.* Developmental expression of ACE2 in the SHR kidney: a role in hypertension? *Kidney Int* 2006; **69**: 34–41.

2. Crackower MA, Sarao R, Oudit GY *et al.* Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 2002; **417**: 822–828.
3. Donoghue M, Hsieh F, Baronas E *et al.* A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res* 2000; **87**: E1–E9.
4. Tipnis SR, Hooper NM, Hyde R *et al.* A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000; **275**: 33238–33243.
5. Vickers C, Hales P, Kaushik V *et al.* Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem* 2002; **277**: 14838–14843.
6. Li N, Zimpelmann J, Cheng K *et al.* The role of angiotensin converting enzyme 2 in the generation of angiotensin 1–7 by rat proximal tubules. *Am J Physiol Renal Physiol* 2004; **288**: F353–F362.
7. Ferrario CM, Jessup JA, Gallagher PE *et al.* Effects of renin angiotensin system blockade on renal angiotensin-(1–7) forming enzymes and receptors. *Kidney Int* 2005; **68**: 2189–2196.
8. Rivière G, Michaud A, Breton C *et al.* Angiotensin-converting enzyme 2 (ACE2) and ACE activities display tissue-specific sensitivity to undernutrition-programmed hypertension. *Hypertension* 2005; **46**: 1169–1174.
9. Chappell MC, Modrall JG, Diz DI, Ferrario CM. Novel aspects of the renal renin-angiotensin system: angiotensin-(1–7), ACE2 and blood pressure regulation. In: Suzuki H, Saruta T (eds). *Kidney and Blood Pressure Regulation*. Karger: Basel, 2004, pp 77–89.
10. Chappell MC, Pirro NT, Sykes A, Ferrario CM. Metabolism of angiotensin-(1–7) by angiotensin converting enzyme. *Hypertension* 1998; **31**: 362–367.
11. Ferrario CM, Trask AJ, Jessup JA. Advances in the biochemical and functional roles of angiotensin converting enzyme 2 and angiotensin-(1–7) in the regulation of cardiovascular function. *Am J Physiol Heart Circ Physiol* 2005; **289**: H2281–H2290.
12. Allred AJ, Diz DI, Ferrario CM, Chappell MC. Pathways for angiotensin-(1–7) metabolism in pulmonary and renal tissues. *Am J Physiol Renal Physiol* 2000; **279**: F841–F850.
13. Gonzales-Villalobos R, Klassen RB, Allen PL *et al.* Megalin binds and internalizes angiotensin-(1–7). *Am J Physiol Renal Physiol* 2006; **290**: F1270–F1275.
14. Zhuo JL, Imig JD, Hammond TG *et al.* Ang II accumulation in rat renal endosomes during Ang II-induced hypertension: role of AT(1) receptor. *Hypertension* 2002; **39**: 116–121.
15. Huang L, Sexton DJ, Kirsten S *et al.* Novel peptide inhibitors of angiotensin-converting enzyme 2. *J Biol Chem* 2003; **278**: 15532–15540.
16. Gallagher PE, Chappell MC, Tallant EA *et al.* Distinct roles for ANG II and ANG-(1–7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. *Am J Physiol Cell Physiol* 2005; **290**: C420–C426.
17. Ferrario CM, Jessup JA, Chappell MC *et al.* Effect of angiotensin converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin converting enzyme 2. *Circulation* 2005; **111**: 2605–2610.
18. Chappell MC, Ferrario CM. Angiotensin-(1–7) in hypertension. *Curr Opin Nephrol Hypertens* 1999; **88**: 231–235.
19. Pendergrass KD, Averill DB, Ferrario CM *et al.* Differential expression of nuclear AT1 receptors and angiotensin II within the kidney of the male congenic mRen2.Lewis rat. *Am J Physiol Renal Physiol* 2006; 290 F1497–F1506

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Non-invasive assessment of kidney oxygenation: a role for BOLD MRI

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Blood oxygen level-dependent (BOLD) contrast magnetic resonance imaging (MRI) has been applied to investigate kidney oxygenation in human patients. These investigations reflect the progress of radiology from a primarily anatomic discipline to one that provides insight into tissue physiology. In particular, magnetic resonance imaging (MRI) is non-invasive, uses no ionizing radiation, and provides insight into disease development and tissue physiology.

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In this issue, research groups from Northwestern University (Chicago, Illinois, USA) and the University of Berne (Switzerland) report having applied blood oxygen level-dependent (BOLD) contrast magnetic resonance imaging (MRI) to investigate kidney oxygenation in human patients.[1,2] These investigations reflect the progress of radiology from a primarily anatomic discipline to one that provides insight into tissue physiology. Progress in instrumentation, computing power, and data analysis has revolutionized the abilities of radiological techniques to provide non-invasive insight into disease development and tissue physiology. In particular, MRI is non-invasive and uses no ionizing radiation. Modern clinical magnetic resonance scanners can provide exquisite anatomy, but more importantly, magnetic resonance can provide images sensitive to multiple parameters, for example, longitudinal relaxation (T_1 ($=1/R_1$)), transverse relaxation (T_2 ($=1/R_2$) and (T_2^* ($=1/R_2^*$)), and diffusion, with additional separation of water, fat, and metabolite images. These facets make MRI a complex discipline, but they open enormous opportunities for investigating tissue pathophysiology. The application of appropriate spin physics can give insight into tissue perfusion, intracellular water diffusion, blood flow, and oxygenation, which is most pertinent here.

The variation of the water proton nuclear magnetic resonance T_2 with blood oxygenation was first reported by Thulborn *et al.* some 20 years ago.[3] Ogawa *et al.*[4] pioneered the application to tissues, and the BOLD approach has now become a mainstay for interrogating neurological function with so-called functional MRI. The observations are predicated on the paramagnetic properties of deoxyhemoglobin, which induces susceptibility gradients in blood, causing loss of signal in T_2^*-weighted magnetic resonance images. Conversion to oxyhemoglobin leads to signal gain.

A number of investigators[5,6] have shown relationships between R_2 or R_2^* and partial pressure of oxygen (pO_2) in blood, and an example is presented in Figure 1. In the range 4–148 torr, both R_2 and R_2^* are sensitive to pO_2, whereas R_1 is essentially invariant. Ultimately, complex quadratic relationships are often found over the range 0–760 torr, due to the sigmoidal binding of oxygen with hemoglobin. The formation of deoxyhemoglobin directly alters T_2, and this has been successfully applied to estimate pO_2 in major blood vessels or the heart, brain, and abdomen. In other tissues, it can be a little more complicated,

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